RNA isolation

User manual

NucleoSpin® RNA Plus XS

November 2019 / Rev. 04

MACHEREY-NAGEL
www.mn-net.com

RNA isolation
Protocol at a glance (Rev. 04)

		NucleoSpin® RNA Plus XS
1 Homogenize and lyse sample		100 µL LB1 Homogenize 100 µL LB2
2 Remove gDNA and filtrate lysate		100 x *g*, 2 min 11,000 x *g*, 10 s
3 Adjust binding conditions		150 µL BSXS Mix
4 Bind RNA		Load lysate 300 x *g*, 1 min 11,000 x *g*, 10 s
5 Wash and dry silica membrane		1st wash: 100 µL MDB 11,000 x *g*, 10 s 2nd wash: 500 µL WB2 11,000 x *g*, 10 s 3rd wash: 200 µL WB2 < 20,000 x *g*, 2 min
6 Elute RNA		10–20 µL RNase-free H_2O 11,000 x *g*, 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Plus XS		
REF	**10 preps 740990.10**	**50 preps 740990.50**	**250 preps 740990.250**
Lysis Buffer LB1	6 mL	6 mL	30 mL
Lysis Buffer LB2	6 mL	6 mL	30 mL
Binding Solution BSXS	10 mL	10 mL	50 mL
Wash Buffer MDB	10 mL	10 mL	30 mL
Wash Buffer WB2 (concentrate)	6 mL	12 mL	50 mL
RNase-free H_2O	13 mL	13 mL	13 mL
NucleoSpin® gDNA Removal Column XS (yellow ring)	10	50	250
NucleoSpin® RNA Plus XS Column (light blue ring plus collection tube)	10	50	250
Collection Tube (2 mL)	20	100	500
Collection Tube (1.5 mL)	10	50	250
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1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to prepare Wash Buffer WB2, non-denatured ethanol recommended)

Consumables

- 1.5 mL or 2.0 mL microcentrifuge tubes or PCR reaction tubes (to prepare sample lysate)
- Disposable mirco pistill (optional, for sample homogenisation)
- Sterile RNase-free tips
- NucleoSpin® Bead Tubes Type A or C (optional, please see 6.3 Ordering information)

Equipment

- Manual pipettes
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

Note: Additional reducing agents (e.g., ß-mercaptoethanol, DTT, TCEP) are not required for NucleoSpin® RNA Plus XS preparations.

1.3 RNase-free work environment

Kit components have been tested to ensure they are RNase-free. However, a RNase-free working environment is also a critical factor for performing successful RNA isolation and handling. Therefore, general recommendations to avoid RNase contamination should be followed:

- Maintain a separate area, RNAse-free pipettes, and materials when working with RNA.
- Wear gloves when handling RNA and reagents to avoid contact with skin, which is a source of RNases. Change gloves frequently.
- Use sterile RNase-free plastic tubes. Collection Tubes (2 mL, for column flowthrough and 1.5 mL for elution) are provided in the kit. Tubes for lysate preparation have to be supplied by user.
- Use RNase-free water supplied with the kit for elution.
- Keep all kit components sealed when not in use and all tubes tightly closed when possible.

1.4 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Plus XS** kit is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® RNA Plus XS** kit is designed to purify RNA from small amounts of a variety of cell and tissue types. This kit supplies the NucleoSpin® gDNA Removal Column XS, a spin column that quickly and effectively removes genomic DNA without the need of DNase digestion.

One of the most important aspects during the isolation of RNA is to prevent degradation of the RNA. Cells and tissues are first lysed by incubation in a chaotropic ion Lysis Buffer (LB1 + LB2), which immediately inactivates RNases. The lysate is added to the NucleoSpin® gDNA Removal Column XS (yellow rings) to clarify the lysate and to remove contaminating gDNA. After the addition of the Binding Solution BSXS to the flowthrough, the RNA is bound to the **NucleoSpin® RNA Plus XS** Column (light blue rings). Subsequent wash steps remove salts, metabolites, and macromolecular cellular components. High quality RNA is eluted with RNase-free H_2O.

The RNA preparation using **NucleoSpin® RNA Plus XS** kits can be performed at room temperature.

The eluate should be treated with care because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints, and dust. Keep RNA frozen at –20 °C for short term or –70 °C for long term storage to ensure RNA stability.

2.2 Kit specifications

- **NucleoSpin® RNA Plus XS** kits are recommended for the isolation of RNA from small amounts of cultured cells and tissue. The **NucleoSpin® RNA Plus XS** kits allows purification of high quality RNA. The RNA A_{260}/A_{280} ratio generally exceeds 1.9 (measured in TE buffer, pH 7.5).
- The isolated RNA is ready to use in diverse downstream applications.
- RNA isolated with the **NucleoSpin® RNA Plus XS** kit is of high integrity. RIN (RNA Integrity Number) or RQN (RNA Quality Number) of RNA isolated from fresh high quality sample material (e.g., eukaryotic cells, or fresh mouse liver) generally exceeds 8. However, RNA integrity strongly depends on the sample quality and a sufficient RNA concentration.
- RNA molecules isolated with **NucleoSpin® RNA Plus XS** are longer than approximately 100 nucleotides. Thus, the **NucleoSpin® RNA Plus XS** kit provides an enrichment of mRNA and rRNA. RNA isolated with the **NucleoSpin® RNA Plus XS** kits may contain minute amounts of genomic DNA due to carry-over from the NucleoSpin® gDNA Removal Column XS. The probability of DNA detection with PCR increases with:
  1. The number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells.
  2. decreasing PCR amplicon size.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Plus XS
Technology	Two column silica membrane system: 1. XS column for DNA removal 2. XS column for RNA isolation
Format	Mini spin column – XS design
Sample material	< 10^5 cultured cells < 5 mg tissue
Fragment size	>100 nt
Typical yield	10^5 HeLa cells: ca. 500–2000 ng 10^4 HeLa cells: ca. 50–200 ng 10^3 HeLa cells: ca. 5–20 ng 10^2 HeLa cells: ca. 0.5–2 ng 10^1 HeLa cells: ca. 0.05–0.2 ng 1 HeLa cell: ca. 0.005–0.02 ng 5000 µg liver: ca. 300–500 ng 500 µg liver: ca. 300–500 ng 50 µg liver: ca. 100–250 ng 5 µg liver: ca. 25–70 ng 0.5 µg liver: ca. 2,5–8 ng 0.05 µg liver: ca. 0,25–1,2 ng 0.005 µg liver: 0,025–0,2 ng
A_{260}/A_{280}	1.9–2.2*
A_{260}/A_{230}	1.5–2.5*
Typical RIN (RNA Integrity Number)	> 8*
Elution volume	5–30 µL
Preparation time	18 min/6 preps
Binding capacity	110 µg

*Quality ratio determination strongly depends on a sufficient amount of RNA measured. Make sure to use sufficient amount of RNA that has been validated to enable meaningful ratio determination!

2.3 Handling, preparation, and storage of starting materials

The maximum amount of sample material that can be used with the **NucleoSpin® RNA Plus XS** kit depends on type of sample and its RNA and DNA content.

Maximal amount of sample material to be used per preparation (approximate values):

- Eukaryotic cells (e.g., HeLa cells): 10^5 cells
- Animal tissue: 5 mg (wet weight) e.g. liver, kidney, or 1 mg spleen (wet weight)

Sample storage and RNase inhibition

RNases rapidly degrade RNA within the samples if samples are not protected from RNase activity after harvest.

- Use freshly harvested sample for immediate lysis and RNA purification.
- Store samples in lysis buffer after disruption at -70 °C for up to one year, at 4 °C for up to 24 hours or at room temperature for up to several hours. Samples frozen in lysis buffer should be thawed slowly before starting with the isolation of RNA.
- Flash freeze sample in liquid N_2 immediately upon harvest and store at -70 °C. Frozen samples are stable up to 6 months. Mortar and pestle can be used to pulverize the sample in a frozen state. Make sure that the sample does not thaw prior to contact with lysis buffer.
- Submerge and store samples in NucleoProtect® RNA or RNA*later*® stabilization solution. Make sure to allow complete permeation of the sample with the stabilization solution before freezing it. Remove excess stabilization solution from the sample prior to RNA isolation according to the stabilization solution user manual.

Disruption and homogenisation of sample material

Adherent cells

Completely aspirate cell culture medium and immediately add Lysis Buffer LB1 to the cell culture dish. Avoid incomplete removal of the cell culture medium in order to allow full lysis activity of the Lysis Buffer. For lysis vortex cells vigorously.

To trypsinize adherent growing cells:

Aspirate cell culture medium, and add an equal amount of PBS in order to wash the cells. Aspirate PBS. Add 0.1–0.3 % trypsin in PBS and incubate for an appropriate time to detach the cells from the dish surface. After cell detachment, add cell culture medium, transfer cells to an appropriate tube (not supplied), and pellet by centrifugation for 5 min at 300 x *g*. Remove supernatant and continue with the addition of lysis buffer to the cell pellet.

Cells in suspension

Spin down cells for 5 min at 300 x *g*, remove the supernatant completely and directly add Lysis Buffer LB1 to the cell pellet. Vortex vigorously for resuspension and lysis.

Tissue samples

- Disruption can be performed by NucleoSpin® Bead Tubes Type A or C (see ordering information). In combination with a Vortex-Genie® 2 the tubes can be mounted on a MN Bead Tube Holder (see ordering information). Add sample and Lysis Buffer LB1 to the bead tube and agitate for 5 min. Alternatively, the NucleoSpin® Bead Tubes A can be

used in a bead mill. NucleoSpin®Bead Tubes Type C are not recommended to use with a bead mill. This might be necessary for some tissue types (e.g., lung, heart tissue). Optimal time and frequency have to be determined experimentally. It is recommended to reduce the amount of beads in the tube from approximately 400 µL to approximately 70 µL–100 µL beads in order to enable efficient lysate withdrawal from the Bead Tube.

- Microtube pestles can be used to homogenize frozen material or fresh material in Lysis Buffer LB1 directly in a microcentrifuge tube.

For tissue samples the lysate should be clarified by centrifugation for 3 min at 5000 x *g*. The supernatant is ready to use for **NucleoSpin® RNA Plus XS** protocol. For cultivated cells a clarification is typically not necessary.

2.4 Elution procedures

Elution volumes in the range of 5–30 µL are recommended. The default elution volume is 10–20 µL.

2.5 Stability of isolated RNA

Eluted RNA should always be kept on ice during work for optimal stability. Contamination with almost omnipresent RNases (general lab ware, fingerprints, dust) may lead to degradation of isolated RNA. For short term storage, freeze RNA at –20°C, for long term storage freeze at –70°C.

3 Storage conditions and preparation of working solutions

Attention:

Buffers LB1, LB2, and MDB contain chaotropic salt. Wear gloves and goggles!

CAUTION: Buffers LB1, LB2, and MDB contain chaotropic salt which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components should be stored at room temperature (18–25 °C) and are stable for at least one year. Storage at lower temperatures may cause precipitation of salts.

Before starting any **NucleoSpin® RNA Plus XS** protocol, prepare the following:

- **Wash Buffer WB2**: Add the indicated volume of 96–100 % ethanol (see table below) to Buffer WB2 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer WB2 can be stored at room temperature (18–25 °C) for at least one year.
- If desired, aliquot RNase-free water into RNase-free 1.5 mL tubes (not provided) in order to avoid accidental RNase contamination of the water by repeated opening of the bottle.

NucleoSpin® RNA Plus XS			
REF	**10 preps** **740990.10**	**50 preps** **740990.50**	**250 preps** **740990.250**
Wash Buffer WB2 (concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® RNA Plus XS** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**GHS symbol** ***GHS-Symbol***	**Hazard phrases** ***H-Sätze***	**Precaution phrases** ***P-Sätze***
LB1, LB2	Guanidinium thiocyanate 30–45 % *Guanidinthiocyanat 30–45 %* CAS 593-84-0	 WARNING *ACHTUNG*	302, 412	264W, 273, 301+312, 330
MDB	Ethanol 5–10 % *Ethanol 5–10 %* CAS 64-17-5d	 WARNING *ACHTUNG*	226	210

Hazard phrases

H 226 Flammable liquid and vapor.
Flüssigkeit und Dampf entzündbar.

H 302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

H 412 Harmful to aquatic life with long lasting effects.
Schädlich für Wasserorganismen, mit langfristiger Wirkung.

Precaution phrases

P 210 Keep away from heat/sparks/open flames/hot surfaces. No smoking.
Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.

P 264 Wash with water thoroughly after handling
Nach Gebrauch mit Wasser gründlich waschen

P 273 Avoid release to the environment.
Freisetzung in die Umwelt vermeiden.

P 301+312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.

P 330 Rinse mouth.
Mund ausspülen.

5 NucleoSpin® RNA Plus XS Protocol

Before starting the preparation:

- Check if Wash Buffer WB2 was prepared according to section 3.

1 Homogenize and lyse sample

100 µL LB1

Homogenize

100 µL LB2

Disrupt and clarify the sample according to one of the methods described in section 2.3 by using **100 µL Lysis Buffer LB1.**

Cells

Add LB1 and vortex vigorously.

Tissue

Homogenize in presence of LB1 by using microtube pestle or bead beating.

Clarify tissue lysates by centrifugation for **3 min** at **5000 x *g***.

Note: Lysis tube is not included in the kit. Addition of reducing agent (e.g., ß-mercaptoethanol, DTT, or TCEP) is not necessary.

Add **one volume Lysis Buffer LB2** to the lysate (typically 100 µL) and mix.

Note: 100 µL LB1 is sufficient for lysis of samples < 20 µL in volume (e.g., 1 mg tissue or low titer cell suspensions). For processing of larger samples (e.g., cell suspensions of > 20 µL) or if a larger lysate volume is desired (e.g., for bead beating), increase the volumes of Lysis Buffer LB1, LB2, and Binding solution BSXS proportionally. Be aware that 180 µL LB1 (+ 180 µL LB2 + 270 µL BSXS) should not be exceeded due to the restricted volume of the column and collection tube.

2 Remove gDNA and filtrate lysate

100 x *g*, 2 min

11,000 x *g*, 10 s

Place a **NucleoSpin® gDNA Removal Column XS** (yellow ring) in a Collection Tube (2 mL, provided), **transfer** the homogenized **lysate** (~200 µL) to the column, and centrifuge for **2 min** at **100 x *g*** followed by **10 s** at **11,000 x *g***.

Discard the column and **continue with the flowthrough**.

Note: Ensure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane. The low-g centrifugation allows efficient DNA removal.

3 Adjust RNA binding conditions

Add **150 µL Binding Solution BSXS** (1.5 volumes relative to LB1) to the flowthrough and mix well by moderate vortexing or by pipetting up and down several times.

150 µL BSXS

Mix

Note: If mixing is done by vortexing, be careful in order to avoid spilling, because the Collection Tube does not contain a lid.

4 Bind RNA

Transfer the whole **lysate** (~350 µL) to the **NucleoSpin® RNA Plus XS Column** (light blue ring) preassembled with a Collection Tube.

load lysate

300 x *g*, 1 min

Centrifuge for **1 min at 300 x *g*** followed by **10 s** at **11,000 x *g***.

Note: The flowthrough may remain in the collection tube.

11,000 x *g*, 10 s

5 Wash and dry silica membrane

1st wash

Add **100 µL Wash Buffer MDB** onto the column.

100 µL MDB

Centrifuge for **10 s** at **11,000 x *g***.

11,000 x *g*, 10 s

Discard the flowthrough with collection tube and place the column into a new 2 mL Collection Tube (provided).

2nd wash

Add **500 µL Buffer WB2** onto the column.

500 µL WB2

Centrifuge for **10 s** at **11,000 x *g***.

Discard the flowthrough and reuse the collection tube.

11,000 x *g*, 10 s

3rd wash

Add **200 µL Buffer WB2** onto the column.

200 µL WB2

Centrifuge for **2 min** at **full speed** (<20,000 x *g*) to dry the membrane.

Place the column into a **Collection Tube** (1.5 mL, provided).

<20,000 x *g*, 2 min

Note: If for any reason, the liquid level in the Collection Tube has reached the column after centrifugation, discard flowthrough and centrifuge again.

6 Elute RNA

Add **10–20 µL RNase-free H_2O** and centrifuge **1 min** at **11,000 x *g***.

Note: Elution volume may be varied from 5 µL to 30 µL.

10–20 µL RNase-free H_2O

11,000 x *g*, 1 min

6 Appendix

6.1 Removal of DNA

In case samples with high initial DNA content are analyzed by downstream applications highly sensitive towards DNA contamination, an additional DNA digest might be required. The protocol for DNase treatments is given below.

Protocol A: DNA digestion in solution

1 Digest DNA (Reaction setup)

Add **1 µL Reaction Buffer for rDNase** and **0.1 µL rDNase** to **10 µL** eluted RNA.

Note: Alternatively, premix 100 µL Reaction Buffer for rDNase and 10 µL rDNase and add 1/10 volume to one volume of RNA eluate.

Gently swirl the tube in order to mix the solution. Spin down gently (approx. 1 s at 1,000 x *g*) to collect every droplet of the solution at the bottom of the tube.

2 Incubate sample

Incubate for **10 min** at **37 °C**.

3 Repurify RNA

Repurify RNA with a suitable RNA clean up procedure, for example by use of the NucleoSpin® RNA Clean-up or NucleoSpin® RNA Clean-up XS kits (please see 6.3 Ordering information), or by ethanol precipitation.

Ethanol precipitation, exemplary:

Add **0.1 volume** of **3 M sodium acetate, pH 5.2** and **2.5 volumes of 96–100 % ethanol** to **one volume of sample**. Mix thoroughly.

Incubate **several minutes** to **several hours** at **–20 °C or 4 °C**.

Note: Choose long incubation times if the sample contains low RNA concentration. Short incubation times are sufficient if the sample contains high RNA concentration.

Centrifuge for **10 min** at **maximum speed.**

Wash RNA pellet with 70 % ethanol.

Dry RNA pellet and resuspend RNA in RNase-free H_2O.

Protocol B: On column DNA digestion

1 Reconstitution of rDNAse

Add 4 mL Reaction Buffer for rDNase into a rDNAse Vial Size F and dissolve the DNase.

2 On-Column digestion

Follow the purification procedure according to section 5 until the column has been washed with 100 µL Wash Buffer MDB (step 5).

Apply **95 µL rDNase reaction mixture** directly onto the center of the silica membrane of the column.

Incubate at room temperature for 15 min.

Continue the procedure 5.1, step 5, by adding 200 µL Buffer WB2 onto the column.

6.2 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/no RNA obtained	*RNase contamination* • Create an RNase free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use. *Insufficient sample quality* • Control sample harvest, storage and lysis. Make sure that samples are harvested, stored, and lysed adequately in order to preserve RNA integrity. See section 2.3 for recommended procedures.
Poor RNA quality or yield	*Reagents not applied or restored properly* • Reagents not properly restored. Add the indicated volume of 96 % ethanol to Buffer WB2 Concentrate and mix. • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. • No Binding Solution BSXS has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of Binding Solution. *Kit storage* • Store kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination.

Poor RNA quality or yield (continued)	*Ionic strength and pH influence A_{260} absorption as well as ratio A_{260}/A_{280}* For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also: • Manchester, K L. 1995. Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. Biotechniques 19, 208–209. • Wilfinger, W W, Mackey, K and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22, 474–481. *Sample material* • Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N_2. Samples should always be kept at -70 °C. Never allow tissues to thaw before addition of Lysis Buffer LB1. Perform disruption of samples in liquid N_2. Alternatively, RNA stabilization solutions may be used to protect RNA from degradation (e.g., NucleoProtect® RNA; see ordering information). • Insufficient disruption and / or homogenization of starting material. Ensure thorough sample disruption.
Low A_{260} / A_{230} ratio	*Carry-over of guanidinium thiocyanate* • Carefully load the lysate to the NucleoSpin® RNA Plus XS Column and try to avoid a contamination of the upper part of the column and the column lid. • Make sure that a sufficient amount / concentration of RNA is used for quantification so that the A_{230} value is significantly higher than the background level. • Measurement of low amount / concentration of RNA will cause unstable A_{260}/A_{230} ratio values.
Clogged NucleoSpin® Column / Poor RNA quality or yield	*Sample material* • Too much starting material used. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of lysis buffer. • Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use NucleoSpin® gDNA Removal Column XS for DNA removal and for easy homogenization of disrupted starting material. • Increase *g*-force and centrifugation time if necessary.

Contamination of RNA with genomic DNA	*Too much cell material used* • Reduce quantity of cells or tissue used. *DNA detection system too sensitive* • The amount of DNA contamination is effectively reduced by the NucleoSpin® gDNA Removal Column XS. However, it can not be guaranteed that the purified RNA is 100 % free of DNA, therefore in very sensitive applications it might still be possible to detect DNA. The probability of DNA detection with PCR increases with: - the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells - decreasing of PCR amplicon size. • Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible. • **Use support protocol 6.1 for subsequent rDNase digestion in solution.**
Suboptimal performance of RNA in downstream experiments	*Carry-over of ethanol or salt* • Do not let the flowthrough touch the column outlet after the second Buffer WB2 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer WB2 completely. • Check if Buffer WB2 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer WB2. *Store isolated RNA properly* • Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at –20 °C, for long term storage freeze at –70 °C.
Too much gDNA contamination	*Too much sample material* • A: Use 150 µL LB1 and 50 µL LB2 instead of a 1:1 ratio. This will reduce gDNA further, but RNA yield will decrease. However, the RNA to DNA ratio will improve. • B: Perform an DNA digestion in solution or on column according to section 6.1 *Store isolated RNA properly*
No RNA yield	*LB2 not applied* • For optimal results, lysis shoud be performed in LB1 and then the lysate should be mixed with on volume LB2. If only LB1 is used, DNA and RNA will bind to the NucleoSpin® gDNA Removal Column XS

6.3 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Plus XS	740990.10 / .50 / .250	10 / 50 / 250
NucleoSpin® RNA Plus	740984.10 / .50 / .250	10 / 50 / 250
NucleoZOL	740404.200	200 mL
NucleoSpin® RNA Set for NucleoZOL	740406.50	50
NucleoSpin® RNA Clean-up	740948.10 / .50 / .250	10 / 50 / 250
NucleoSpin® RNA Clean-up XS	740903.10 / .50 / .250	10 / 50 / 250
rDNase Set	740963	1
Collection Tubes	740600	1000
NucleoSpin® Bead Tube Type A	740786.50	50
NucleoSpin® Bead Tube Type C	740813.50	50
MN Bead Tube Holder	740469	1
NucleoProtect® RNA	740400.50 / .250 / .500	50 / 250 / 500 mL

6.4 Product use restriction / warranty

NucleoSpin® RNA Plus XS kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

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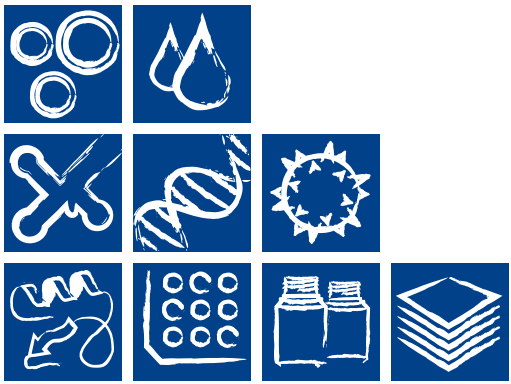


Plasmid DNA

Clean up

RNA

DNA

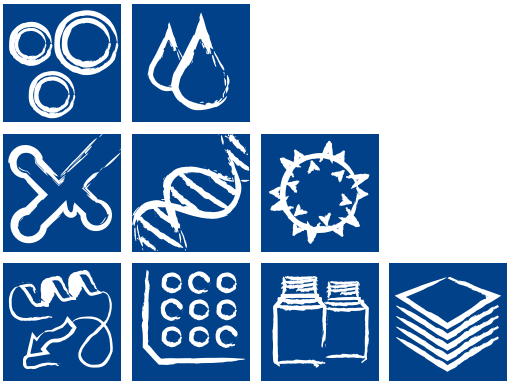
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



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